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APPLICATION NUMBER: *60/536,493*
FILING DATE: *January 15, 2004*

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No.

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
1) Ilan	ZIV	Kfar Saba, ISRAEL

Additional inventors are being named on the 2nd *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

PERTURBED MEMBRANE-BINDING COMPOUNDS

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 47
☑ Drawing(s) *Number of Sheets* 1
☐ Application Date Sheet. See 37 CFR 1.76
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Respectfully submitted,

SIGNATURE Lee C. Heiman

TYPED or PRINTED NAME Lee C. Heiman

TELEPHONE 202-775-8383

Date January 15, 2004

REGISTRATION NO. 41,827 *(if appropriate)*

Docket Number: 25934

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Docket Number 25934

INVENTOR(S)/APPLICANT(S)

Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)
2) Anat	SHIRVAN	Herzliya, ISRAEL

Number 2 of 2

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MAIL STOP PROVISIONAL PATENT APPLICATION
Attorney Docket No. 25934

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

ZIV et al.

Serial No. NOT YET ASSIGNED

Filed: January 15, 2004

For: **PERTURBED MEMBRANE-BINDING COMPOUNDS**

TRANSMITTAL LETTER

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Submitted herewith for filing in the U.S. Patent and Trademark Office is the following **PROVISIONAL APPLICATION:**

(1) Transmittal Letter
(2) Cover sheet for filing **Provisional Application**
(3) 48 page Provisional Application consisting of:
34 pages Textual Specification,
12 pages of 36 Claims,
1 page of the Abstract,
1 sheet of Drawings;
(4) Check No. 20172 $ 80.00 for filing fee as a small entity;
(5) Postcard for early notification of serial number.

The Commissioner is hereby authorized to charge any deficiency or credit any excess to Deposit Account No. 14-0112.

Respectfully submitted,
NATH & ASSOCIATES PLLC

By: Lee C. Heiman

Gary M. Nath
Registration No. 26,965
Lee C. Heiman
Registration No. 41,827
Customer No. 20529

Date: January 15, 2004
NATH & ASSOCIATES PLLC
1030th 15TH Street, NW - 6th Floor
Washington, D.C. 20005
GMN/LCH/dd:APPL.trans

PERTURBED MEMBRANE-BINDING COMPOUNDS

FIELD OF THE INVENTION

The invention relates to compounds that selectively bind to cells undergoing perturbations and alterations of their normal plasma membrane organization, i.e., cells undergoing cell death, apoptotic cells or activated platelets. The invention further provides methods for utilizing said compounds in medical practice, for diagnostic and therapeutic purposes.

BACKGROUND OF THE INVENTION

The plasma membrane (outer membrane) of intact eukaryotic cells is characterized by a highly organized structure. This high level of membrane organization is determined, among others, by the molecular structure of the specific lipids constituting the membrane; the ratio between the various lipid species from which the membrane is composed; the distribution of the phospholipids between the outer and inner leaflets of the membrane; and by the membrane protein constituents.

While maintenance of the high level of plasma membrane organization is fundamental to normal cell physiology, substantial *p*erturbations and alterations of the *n*ormal *o*rganization of the cell plasma *m*embrane (PNOM) occur in numerous physiological and pathological conditions, and are characterizing a plurality of diseases. Such alterations and perturbations may be evident both at the morphological level (membrane blebbing observed in cells undergoing apoptosis) and at the molecular level. The scope of perturbations accompanying either cell death, cell disease or cell activation, is not fully elucidated. They include, among others, scrambling and redistribution of the membrane phospholipids, with

movement to the cell surface of aminophsopholipids, mainly phosphatidylserine (PS) and phosphatidylethanolamine (PE), which are normally restricted almost entirely to the inner leaflet of the membrane bilayer, and reciprocal movement of sphingomyelin (SM) and phosphatidylcholine (PC) from the outer leaflet to the inner leaflet of the membrane. This redistribution is referred herein as loss of *c*ell *m*embrane *l*ipid *a*symmetry (CMLA). In addition to PNOM, CMLA loss is also often associated with reduction in the level of packing of membrane phospholipids and an increase in membrane fluidity.

These alterations play an important role in making the cell surface a catalytic platform for the assembly of several clotting factor complexes, such as the tenase and prothrombinase protein complexes. Accordingly, platelet activation is associated with platelet membrane undergoing PNOM, and these alterations constitute an important factor in normal blood coagulation, as well as in the initiation and/or propagation of abnormal, excessive blood clotting in numerous disorders. These disorders include, among others, arterial or venous thrombosis or thrombo-embolism [e.g., cerebral stroke, myocardial infarction, deep vein thrombosis (DVT), disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura, etc.], unstable atherosclerotic plaques, sickle cell disease; beta-thalassemia, anti-phospholipid antibody syndrome [among others in systemic lupus erythematosus (SLE)], and disorders associated with shedding of membrane microparticles, e.g., neurological dysfunction in association with cardiopulmonary bypass.

Apoptosis is another major situation in which alterations / perturbations of cell membrane take place. Apoptosis is an intrinsic program of cell self-destruction or *"suicide"*, which is inherent in every eukaryotic cell. In response to a triggering stimulus, cells undergo a highly characteristic cascade of events of cell shrinkage, blebbing of cell membranes, chromatin condensation and fragmentation, culminating in cell conversion to clusters of membrane-bound particles (apoptotic bodies), which are thereafter engulfed by macrophages. PNOM is a universal phenomenon of apoptosis, it occurs early in the apoptotic cascade, probably at the point of cell

commitment to the death process, and has also been shown to be an important factor in the recognition and removal of apoptotic cells by macrophages.

A strong correlation has been recently drawn between PNOM and a potent procoagulant activity of apoptotic cells. PNOM in apoptotic endothelial cells, such as those occurring in atherosclerotic plaques, probably plays an important role in the pathogenesis of thrombotic vascular disorders.

Since apoptosis or thrombosis has an important role in the majority of medical disorders, it is desirable to have tools for detection of these biological processes and targeting of associated cells. Compounds for selective binding to PNOM membranes, potentially also performing subsequent entry into these cells having such PNOM membranes (PM cells), may therefore serve as an important tool for detecting and targeting of cells undergoing damage or death process, especially by apoptosis, or platelets undergoing activation.

In the clinical context, targeting of cells having PNOM membranes may be useful in at least the following distinct aspects of medical practice:

(i). *Diagnostics:* Diagnosis of disease, monitoring of the course or progression of a disease, or monitoring the effect of various therapeutic approaches utilized to alter disease course.

(ii). *Therapeutics:* Targeting of therapeutic agents, which may be agents for preventing, treating or ameliorating the disease, to foci of disease, characterized by cell death, especially by apoptosis, and /or thrombosis, in order to allow and enhance the modulation of said disease processes by said agents.

(iii). *Clearance:* Clearance of pro-coagulant elements, such as apoptotic cells or activated platelets from body fluids, e.g., blood.

SUMMARY OF THE INVENTION

In an embodiment of the invention, there are provided compounds that can selectively bind to cells undergoing *p*erturbation of their *n*ormal *o*rganization of the plasma *m*embrane (PNOM-cells), while binding to a much lesser degree, namely at least two fold less, to cells, which maintain the normal organization of their plasma membrane. The PNOM-cells are selected from cells undergoing a death process, apoptotic cells and activated platelets. The invention further relates to methods of detecting PNOM-cells by using these compounds, which selectively bind to the PNOM cells. In another embodiment of the invention, the invention is related to novel compounds which may be represented by any of the structure set forth in formulae I-VII.

The term *"perturbed membrane-binding compound"* (PMBC) refers to a compound that performs selective targeting to PNOM-cells, while binding to a lesser degree (at least two fold less) to normal cells.

In an aspect of the invention, the compounds of the invention The term *"selective targeting"* refers in the invention to selective binding of a compound to PNOM-cells, while binding to a lesser degree (at least two fold less) to normal cells.

The term *"diagnostic perturbed membrane-binding compound"* (diagnostic PMBC) refers to a compound capable of selective targeting to PNOM cells, wherein said compound comprises a marker, said marker being detectable by means known to those skilled in the art.

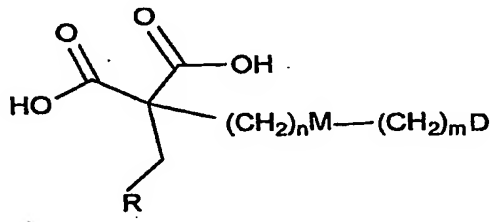
The term *"therapeutic perturbed membrane-binding compound"* (therapeutic PMBC) refers to a PMBC as defined above, comprising a drug, useful in the treatment of disease.

The term *"solid support"* refers in the contents of the present invention to a solid matrix, an insoluble matrix, and an insoluble support. The solid support in accordance with the present invention may be formed in a variety of structures such as a stack of micro-particulates, micro-filters, or micro-capillara, and may be

composed of various materials such as alumina, diatomaceous earth, celite, calcium carbonate, calcium sulfate, ion-exchange resin, silica gel, charcoal, amberlite, dowex, Eupergit and ethylsofoxycellulose.

The PMBC is used in an embodiment of the invention for the preparation of an agent for selective targeting cells, which have perturbed membranes.

(i) In one aspect, the present invention provides a compound which selectively binds to PNOM- cells, represented by the structure set forth in formula (I):



(I)

or pharmaceutically acceptable salts, metal chelates, solvates and hydrates of the compound represented by the structure as set forth in formula (I), and solvates and hydrates of said salts; wherein,

R represents hydrogen, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10} linear or branched alkyl, linear or branched hydroxy-alkyl; or linear or branched fluoro-alkyl.

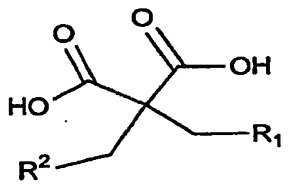
n and m each stands for an integer of 0, 1, 2, 3 or 4; n and m can be the same or different ;

M is selected from null, -O-, -S-, -C(O)NH-, and -NU, wherein U stands for a null, hydrogen, C_1, C_2, C_3, or C_4 alkyl, or C_1, C_2, C_3, or C_4 alkylene;

D is selected from hydrogen, a drug to be targeted to the PNOM cell; and a marker for diagnostics selected from a marker for imaging and a metal chelate; said marker for imaging being selected from the group comprising a fluorescent label, a radio-label, a marker for X-ray, a marker for MRI, a marker for PET scan and a label capable of undergoing an enzymatic reaction that produces a detectable color, and

where the above alkylene groups in formula (I) bound to M or D may be each substituted at each occurrence by a group selected from amino, F, OH and SH.

In another embodiment of the invention there is provided a compound represented by the structure as set forth in formula (II):

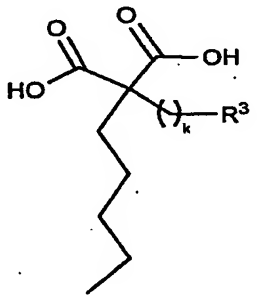


II

including pharmaceutically acceptable salts hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (II) and solvates and hydrates of said salts; wherein R^1 is hydrogen or C_1, C_2, C_3, C_4, or C_5 linear or branched alkyl, and R^2 is hydrogen or C_1, C_2, C_3, C_4, or C_5 linear or branched alkyl, hydroxy-alkyl or fluoro-alkyl.

In another embodiment of the invention, there is provided a compound represented by the structure as set forth in formula (II) including pharmaceutically acceptable salts hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (II) and solvates and hydrates of said salts; wherein R^1 is hydrogen or C_1, C_2, C_3, C_4, or C_5 linear or branched alkyl, and R^2 is C_1, C_2, C_3, C_4, or C_5 fluoro-alkyl.

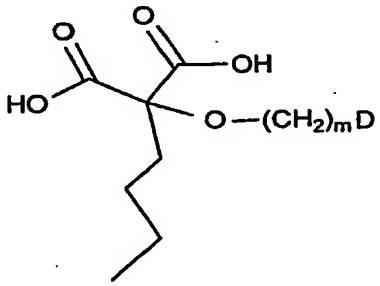
In another embodiment of the invention there is provided a compound represented by the structure as set forth in formula (III):



III

including pharmaceutically acceptable salts hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (II) and solvates and hydrates of said salts; wherein R^3 is hydroxyl or F and k is an integer selected from 1, 2, 3, 4 and 5.

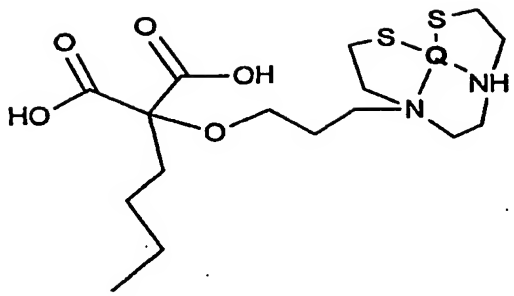
In another embodiment of the invention there is provided a compound represented by the structure as set forth in formula (IV):



IV

including pharmaceutically acceptable salts hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (III) and solvates and hydrates of said salts, wherein m and D are as defined above.

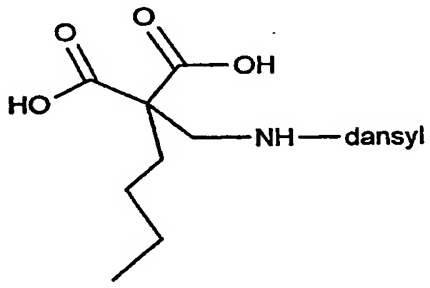
In another embodiment of the invention there is provided a compound represented by the structure set forth in formula V:



(V)

including pharmaceutically acceptable salts hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (IV) and solvates and hydrates of said salts, wherein m and Q is a metal atom selected from Technetium, oxo-Technetium, Rhenium and oxo-Rhenium.

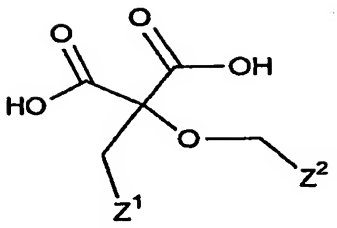
In another embodiment of the invention there is provided a compound represented by the structure as set forth in formula (VI), and designated NST-ML-D:



VI

including pharmaceutically acceptable salts hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (VI) and solvates and hydrates of said salts.

In another embodiment of the invention there is provided a compound represented by the structure as set forth in formula (VII):



VII

including pharmaceutically acceptable salts hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (VII) and solvates and hydrates of said salts; wherein Z^1 and Z^2 are each selected from hydrogen and C_1, C_2, C_3, C_4, C_5 alkyl, hydroxy-alkyl or fluoro-alkyl; Z groups may be the same or different.

In another aspect of the invention, there is provided a pharmaceutical composition for targeting of drugs to foci of apoptosis or blood clotting in a patient, where said patient is a human or non-human mammal, said pharmaceutical composition comprising a compound according to the structure set forth in formulae I-VII, wherein said compound comprises or is being linked to a drug.

In an aspect of the invention, there is provided a method of selectively targeting a medicinally-useful compound to a PNOM-cell, said method comprising:

(i). contacting a cell with a compound represented by the structure set forth in any one of formulae I-VI:

(ii). thereby selectively targeting the medicinally-useful compound to the PNOM-cell.

In another aspect of the invention, there is provided a method of detecting a PNOM-cell within a cell population, said method comprising:

(i) contacting the cell population with compound represented by the structure set forth represented by the structure set forth in any one of formulae I-VII: or pharmaceutically acceptable salts, metal chelates, solvates and hydrates of the

compound represented by the structure as set forth in any one formulae I-VII and solvates and hydrates of said salts; wherein D is a marker for diagnostics and R and M are as defined above;

(ii) determining the amount of said compound bound to said cells, wherein a significant amount of said compound bound to a cell indicates its being a PNOM-cell.

In another aspect of the invention, there is provided a method for detecting of PNOM-cells in a patient or an animal, the method comprising:

(i) administering a compound to the patient or animal represented by the structure set forth in formulae I-VII, or pharmaceutically acceptable salts, metal chelates, solvates and hydrates of the compound represented by the structure as set forth in formulae I-VII and solvates and hydrates of said salts wherein D is a marker for diagnostics and R and M are as defined above; and

(ii) (ii) imaging the examined patient or animal, so as determine the amount of the compound bound to cells, wherein a significant amount of compound bound a cell indicates its being a PNOM-cell.

In another embodiment of the invention, there is provided a compound represented by the structure as set forth in formulae I-VII or pharmaceutically acceptable salts, metal chelates, solvates and hydrates of the compound represented by the structure as set forth in formulae I-VII, and solvates and hydrates of said salts; Wherein M and R are as defined above.

In another aspect of the invention, there is provided a pharmaceutical composition for targeting of drugs to foci of apoptosis or blood clotting in a patient, where said patient is a human or non-human mammal, said pharmaceutical composition comprising a compound according to the structure set forth in formulae I-VII, wherein said compound comprises or is being linked to a drug.

BRIEF DESCRIPTION OF THE DRAWINGS

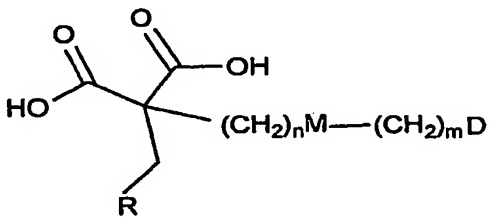
Fig. 1: Scheme of the mechanism of action of the compounds of the invention: the NST-ML-Action Motif.

DETAILED EMBODIMENTS OF THE INVENTION

The present invention is related to compounds, which can in one embodiment selectively bind to cells undergoing *p*erturbation of their *n*ormal *o*rganization of their plasma *m*embrane (PNOM-cells), while binding to a much lesser degree to cells maintaining the normal organization of their plasma membrane. The PNOM-cells are selected from cells undergoing a death process, apoptotic cells and activated platelets. The invention further relates to methods of detecting PNOM-cells by using compounds, which selectively bind to the PNOM-cells.

The compounds of the invention have the advantage of being active in performing selective targeting of PNOM cells, while also featuring a relatively low molecular weight, and a potentially favorable pharmacokinetic profile, including, for example, reduced binding to albumin.

In one embodiment, there is provided a compound which selectively targets to a PNOM cell having the structure set forth in formula (I):



(I)

or pharmaceutically acceptable salts, metal chelates, solvates and hydrates of the compound represented by the structure as set forth in formula (I), and solvates and hydrates of said salts; wherein,

R represents hydrogen, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10} linear or branched alkyl, linear or branched hydroxy-alkyl; or linear or branched fluoro-alkyl.

n and m each stands for an integer of 0, 1, 2, 3 or 4; n and m can be the same or different ;

M is selected from null, -O-, -S-, -C(O)NH-, and -NU, wherein U stands for a null, hydrogen, C_1, C_2, C_3, or C_4 alkyl, or C_1, C_2, C_3, or C_4 alkylene;

D is selected from hydrogen, a drug to be targeted to the PNOM cell and a marker for diagnostics selected from a marker for imaging and a metal chelate; the marker for imaging may be detected by color, fluorescence, x-ray, CT scan, magnetic resonance imaging (MRI) and radio-isotope scan such as single photon emission tomography (SPECT) or positron emission tomography (PET);
and where the above alkylene groups bound to M or D may be each substituted at each occurrence by a group selected from amino, F, OH and SH.

The drug may be a medicinally-useful agent for the prevention, amelioration, or treatment of a specific disease and may be, for example, without being limited:

(i) An inhibitor of apoptosis, (e.g., a caspase inhibitor, antioxidant, modulator of the Bcl-2 system);

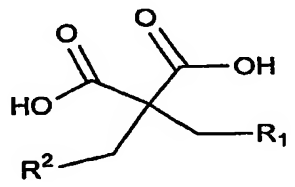
(ii) An activator of cell death (e.g. an anticancer drug);

(iii) A modulator of blood coagulation, which may be an anticoagulant, an antithrombotic, or a thrombolytic agent. In such case, said drug is preferably selected among an antiplatelet agent, heparin, low molecular weight heparin, antagonists of glycoprotein IIb/IIIa, tissue plasminogen activator (tPA), or an inhibitor of a clotting factor, such as an inhibitor of thrombin or an inhibitor of factor Xa; or

(iv) An anti-inflammatory drug or an immuno-modulatory drug.

In another embodiment of the invention, D may be a solid support.

In another embodiment of the invention there is provided a compound which selectively targets a PNOM cell represented by the structure as set forth in formula (II):

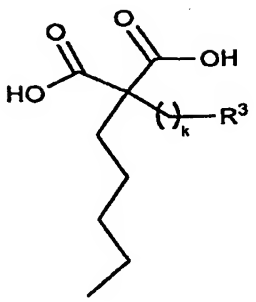


II

including pharmaceutically acceptable salts hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (II) and solvates and hydrates of said salts; wherein R^1 is hydrogen or C_1, C_2, C_3, C_4, or C_5 linear or branched alkyl, and R^2 is hydrogen or C_1, C_2, C_3, C_4, or C_5 linear or branched alkyl, hydroxy-alkyl or fluoro-alkyl.

In another embodiment of the invention, there is provided a compound represented by the structure as set forth in formula (II) including pharmaceutically acceptable salts hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (II) and solvates and hydrates of said salts; wherein R^1 is hydrogen or C_1, C_2, C_3, C_4, or C_5 linear or branched alkyl, and R^2 is C_1, C_2, C_3, C_4, or C_5 fluoro-alkyl.

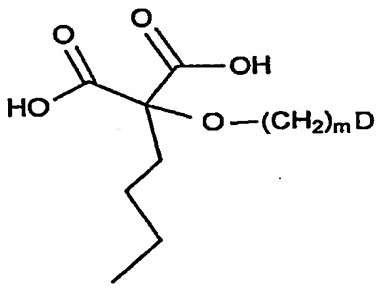
In another embodiment of the invention there is provided a compound represented by the structure as set forth in formula (III):



III

including pharmaceutically acceptable salts hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (II) and solvates and hydrates of said salts; wherein R^3 is hydroxyl or F and k is an integer selected from 1, 2, 3, 4 and 5..

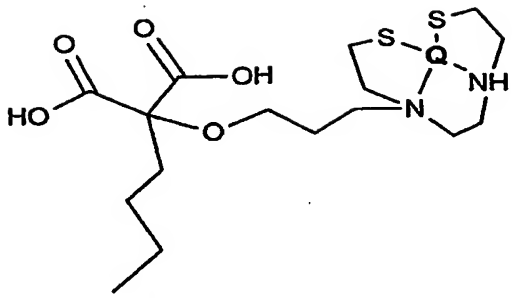
In another embodiment of the invention there is provided a compound represented by the structure as set forth in formula (IV):



IV

including pharmaceutically acceptable salts hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (III) and solvates and hydrates of said salts, wherein m and D are as defined above.

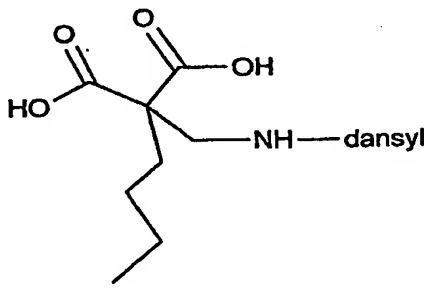
In another embodiment of the invention there is provided a compound represented by the structure set forth in formula V:



(V)

including pharmaceutically acceptable salts hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (IV) and solvates and hydrates of said salts, wherein m and Q is a metal atom selected from Technetium, oxo-Technetium, Rhenium and oxo-Rhenium.

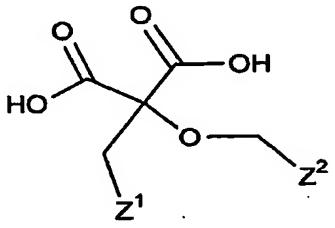
In another embodiment of the invention there is provided a compound represented by the structure as set forth in formula (V), and designated NST-ML-D:



V

including pharmaceutically acceptable salts hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (V) and solvates and hydrates of said salts.

In another embodiment of the invention there is provided a compound represented by the structure as set forth in formula (VI):



VI

including pharmaceutically acceptable salts hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (VI) and solvates and hydrates of said salts; wherein Z^1 and Z^2 are each selected from hydrogen and C_1, C_2, C_3, C_4, C_5 alkyl, hydroxy-alkyl or fluoro-alkyl; Z groups may be the same or different.

In another aspect of the invention, there is provided a method of detecting a PNOM-cell within a cell population, said method comprising:

(i) contacting the cell population with a compound represented by any one of the structure set forth in formulae I-VII or pharmaceutically acceptable salts, metal chelates, solvates and hydrates of the compound represented by the structure as set forth in formulae I-VII and solvates and hydrates of said salts; wherein D is a marker for diagnostics and R and M are as defined above;

(ii) determining the amount of the compound bound to said cells, wherein a significant amount of compound bound to a cell indicates its being a PNOM-cell.

In another aspect of the invention, there is provided a method for detecting of PNOM-cells in a patient or an animal, the method comprising:

(i) administering a compound to the patient or animal a compound represented by the structure set forth in formulae I-VII, or pharmaceutically acceptable salts, metal chelates, solvates and hydrates of the compound represented

by the structure as set forth in formulae I-VII and solvates and hydrates of said salts wherein D is a marker for diagnostics and R and M are as defined above; and

(ii) imaging the examined patient or animal, so as determine the amount of compound bound to cells, wherein a significant amount of compound bound a cell indicates its being a PNOM-cell.

The property of the compounds of the invention, which are capable of selective binding to PNOM-cells, while binding to a much lesser degree, (i.e., at least two fold less) to normal cells, which are referred hereto to cells having plasma membrane of normal organization, may be used for selective targeting of medicinally-useful agents to tissues and organs comprising PNOM-cells, in three different approaches of the invention:

According to a first approach, termed hereinafter the *"detection approach"* said selective binding may be utilized to targeting a marker for imaging to PNOM-cells. This may be used in clinical practice, either *in vivo*, *ex vivo* or *in vitro*, for the diagnosis of diseases in which such cells emerge as will be explained herein below.

According to a second approach, termed hereinafter the *"therapeutic approach"*, said property of selective binding is used for selective targeting of therapeutic agents to organs and tissues in the body wherein PNOM-cells emerge, e.g., regions of cell death, thrombus formation or inflammation.

In accordance with a third approach of the invention termed the *"clearance approach"*, the selective binding of the compounds of the invention to PNOM-cells is utilized, via attachment of said compounds to a solid support, to clear body fluids such as blood from PNOM-cells, which may be potentially hazardous due to their pro-coagulant properties.

Diseases characterized by occurrence of PNOM-cells are diseases which one of their manifestations is occurrence of said cells. This is not meant to read that said PNOM-cells are necessarily the cause, or the sole effect of the disease, but rather that their occurrence are one of its manifestations.

In accordance with the *detection approach*, the present invention concerns a composition comprising as an effective ingredient a PMBC, comprising a marker for imaging, for the detection of PNOM-cells, either *in vitro*, *ex vivo* or *in vivo*. Such a PMBC is hereinafter designated *"diagnostic PMBC"*. The diagnostic PMBC is capable of performing selective binding to PNOM-cells present in the assayed sample. Then, said binding may be identified by any means known in the art. The diagnostic PMBC of the invention enables the targeting of said marker, by the PMBC, to PNOM–cells in a selective manner. Then, the detectable label can be detected by any manner known in the art, and in accordance with the specific label used, for example, fluorescence, radioactive emission, or a color production, MRI, x-ray and the like. The term *"bound"* refers the linkage of the PMBC to the detectable label, said linkage being either a covalent or a non-covalent (e.g., electrostatic) binding.

In one embodiment, the detectable label may be any of the respective radio-isotopes of the metal ions Tc, oxo-Tc, In, Cu, Ga, Xe, Tl and Re, oxo-Re and the covalently linked atoms: ^{123}I and ^{131}I for radio-isotope scan such as SPECT, Gd(III), Fe(III) or Mn(II) for MRI, and ^{18}F, ^{15}O, ^{18}O, ^{11}C, ^{13}C, ^{124}I, ^{13}N and ^{75}Br for positron emission tomography (PET) scan.

In another embodiment, PMBC of the invention is aimed at clinical imaging of apoptosis via PET scan, and the PMBC comprises ^{18}F atom(s).

The attachment of ^{18}F for the purposes of clinical PET imaging may be performed immediately before the administration of the diagnostic compound to the patient. Therefore, it may be useful to synthesize a *PMBC-PET precursor*, comprising a moiety to be substituted by ^{18}F before administration to the patient. In one embodiment, said moiety to be replaced by ^{18}F is selected from a hydroxyl group, a nitro group, or a halogen atom such as Bromine or Chlorine. Such a *PMBC-PET precursor* is also included in the scope of the invention.

The method for labeling a PMBC, which can be any PMBC of the structures described above, with ^{18}F for PET imaging, comprises the step of attaching an ^{18}F

atom to the PMBC; thereby radio-labeling a PMBC with ^{18}F for PET imaging. Optionally, the functional groups of the PMBC may be protected by appropriate protecting groups prior to the step of attaching ^{18}F atom, and are removed after the step of attachment of the ^{18}F atom.

In the case that the marker is a metal atom (e.g., Gd or ^{99m}Tc or oxo-^{99m}Tc, for MRI or SPECT, respectively), the PMBC comprises a metal chelator. The metal coordinating atoms of said chelator may be nitrogen, sulfur or oxygen atoms. In another embodiment of the invention, said chelator is diaminedithiols, monoamine-monoamide-bisthiols (MAMA), triamide-monothiols, and monoamine- diamide-monothiols.

In such case, both the *PMBC-chelate precursor*, being the PMBC attached to or comprising a chelator prior to complexation with the metal atom, and the complex comprising the metal atom are included in the scope of the invention.

For fluorescent detection, the diagnostic PMBC may comprise a fluorescent group such as 5-(dimethylamino) naphthalene-1-sulfonylamide (dansyl-amide).

The compounds of the invention may be used for a detection and diagnosis of a wide variety of medical conditions (including normal conditions, pathological conditions, diseases or disorders which are characterized by formation PNOM-cells.

Examples of conditions characterized by PNOM-cells are as follows:

Diseases which are characterized by occurrence of excessive apoptosis, such as degenerative disorders, neurodegenerative disorders (e.g., Parkinson's disease, Alzheimer's disease, Huntington chorea), AIDS, ALS, Prion Diseases, myelodysplastic syndromes, ischemic or toxic insults, graft cell loss during transplant rejection; tumors, and especially highly malignant / aggressive tumors, are also often characterized by enhanced apoptosis, in addition to the excessive tissue proliferation.

The tumors may be tumors derived, without being limited from the lung, breast or colon.

Diseases manifested by excessive blood clotting, wherein PNOM occurs during platelet activation, and / or during activation of or damage to other cellular elements (e.g., endothelial cells). These diseases include, among others, arterial or venous thrombosis, thrombo-embolism, e.g., myocardial infarction, cerebral stroke, deep vein thrombosis, disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura (TTP), sickle cell diseases, thalassemia, antiphospholipid antibody syndrome, systemic lupus erythematosus.

Inflammatory disorders, and / or diseases associated with immune–mediated etiology or pathogenesis, auto-immune disorders such as antiphospholipid antibody syndrome, systemic lupus erythematosus, connective tissue disorders such as rheumatoid arthritis, scleroderma; thyroiditis; dermatological disorders such as pemphigus or erythema nodosum; autoimmune hematological disorders; autoimmune neurological disorders such as myasthenia gravis; multiple sclerosis; inflammatory bowel disorders such as ulcerative colitis; vasculitis.

Atherosclerotic plaques, and especially plaques that are unstable, vulnerable and prone to rupture, are also characterized by PNOM-cells, such as apoptotic macrophages, apoptotic smooth muscle cells, apoptotic endothelial cells, and activated platelets.

The detection of these pathological conditions, disorders or diseases via detection of the associated PNOM-cells by the diagnostic PMBC of the invention may be an aim by itself, simply for diagnosis of the presence of a disease condition in a specific individual.

Said detection may also be carried out in a person already known to have the disease for the purpose of evaluating the disease severity and in order to monitor disease course and / or response to various therapeutic modalities. An example for such monitoring is evaluation of response to anticancer therapy. Since most anti-tumor treatments, chemotherapy or radiotherapy exert their effect by induction of apoptosis, detection by a diagnostic PMBC of therapy-induced apoptosis of tumor cells may teach on the extent of sensitivity of a tumor to the anti-tumor agent, thus

substantially shortening the lag period between the time of administration of the anti-cancer treatment and the time of proper assessment of its efficacy.

Moreover, said detection may be used also to monitor adverse effects of anti-cancer treatments. A large part of such adverse effects are due to untoward treatment-induced apoptosis in normal, yet sensitive cells, such as those of the gastrointestinal epithelium or the bone marrow hematopoietic system. Detection by the diagnostic PMBC of such apoptosis may allow early detection of this untoward tissue damage and better optimization of the treatment protocol.

In addition, said detection may aim at characterization of intrinsic apoptotic load within a tumor, often correlated with the level of tumor aggressiveness; and may also assist in the detection of metastases, via detection of the intrinsic apoptosis within said metastases.

Similarly, the diagnostic PMBC of the invention may be useful in monitoring graft survival after organ transplantation, since apoptosis plays a major role in cell loss during graft rejection.

In addition, said detection may aim at monitoring response to cyto-protective treatments, and thus aid in screening and development of drugs which are capable of inhibiting cell loss in various diseases (for example those recited above) by enabling a measure of evaluation of cell death.

Said detection may be also useful for the detection of atherosclerotic plaques, since destabilization of such plaques, rendering them vulnerable, prone to rupture, thrombosis and embolization, is characterized by participation of several types of PNOM-cells, including apoptotic cells (apoptotic macrophages, smooth muscle cells and endothelial cells), and activated platelets.

The detection via the diagnostic PMBC of the invention may also take place for basic research purposes in the study of apoptosis in tissue culture and animal models, and may also help in determining the role of apoptosis in normal development and homeostasis of various tissues, such as in the development of the

central nervous system during embryogenesis, as well as during situations such as normal aging.

In accordance with this approach, the present invention is related to a method of detection of PNOM-cells in a cell population, selected from whole body, organ, tissue, tissue culture or any other cell population, said method comprising:

(i) contacting the cell population with a PMBC according to any of the embodiments of the invention;

(ii) determining the amount of PMBC bound to said cell population, wherein detection of a significant amount of compound bound to a cell within said population indicates a PNOM-cell.

In another embodiment, the present invention further relates to a method for detecting of PNOM-cells in a patient or an animal *in vivo*, the method comprising:

(i) administering a diagnostic PMBC to the examined patient or animal; said administration being performed by any means known in the art, such as parenteral (e.g., intravenous) or oral administration;

(ii) imaging the examined patient or animal, by any method known in of the art (e.g., PET scan, SPECT, MRI), to detect and determine the amount of diagnostic PMBC bound to cells, wherein a significant amount of compound bound a cell indicates its being a PNOM-cell.

In another more specific embodiment of the invention, the present invention is related to an method for the detection of PNOM-cells in a tissue or cell culture sample *in vitro* or *ex-vivo*, the method comprising:

(i) contacting said sample with a diagnostic PMBC, which may be any of the PMBC compounds described in the invention under conditions enabling binding of said diagnostic PMBC to biological membranes of PNOM- cells;

(ii) detecting the amount of bound diagnostic PMBC to said cells; the presence of a significant amount of diagnostic bound compound indicating the presence of PNOM-cells.

The step of detection in said *in vitro* or *ex-vivo* studies may be for example, in the case of fluorescent-labeled compound of the invention, without limitation by using flow cytometric analysis, which permits cell visualization on equipment that is widely commercially available. For example, the FACSort flow cytometer (Becton-Dickinson Immunocytometry Systems, San Jose, Calif.) is typically interfaced to a PC, such as the Consort 32 workstation (Hewlett-Packard). In an example using fluorescence to visualize cells, a single 15 mW argon ion laser beam (488 nm) is used to excite the FITC fluorescence, and fluorescence data is collected using 530 nm band pass filter to provide a histogram. The percent of fluorescent cells can be calculated, for example using Lysis II software. The method for detection may be used in an embodiment of the invention for screening therapeutic drugs such as anticancer drugs.

The term "*significant amount*" according to the invention means that the amount of PMBC bound to a PM-cell is at least twice the amount bound to a non-PM-cell. The actual amount may vary according to the imaging method and equipment utilized, and according to the organs or tissues examined. In another embodiment the amount of PMBC bound to a PM-cell is at least four times the amount bound to a non-PM-cell.

In another embodiment the amount of PMBC bound to a PM-cell is at least six times the amount bound to a non-PM-cell.

In another embodiment the amount of PMBC bound to a PM-cell is at least eight times the amount bound to a non-PM-cell.

In another embodiment the amount of PMBC bound to a PM-cell is at least ten times the amount bound to a non-PM-cell.

The amount is depend on the method of measuring said difference in binding.The method of the present invention may be used for the diagnosis of a disease characterized by the occurrence of PNOM-cells, for example, without being limited to any of the diseases mentioned above.

The method of the present invention may also be used for monitoring the effects of various therapeutic modalities for said diseases or medical conditions, or alternatively for basic science research purposes as explained above.

In accordance with a second approach of the invention, termed *"the therapeutic approach"*, the present invention concerns a pharmaceutical composition comprising a PMBC as an active ingredient, optionally with a pharmaceutically acceptable carrier; said PMBC comprising a medicinally-useful, pharmaceutically-active drug. Said PMBC is designated *"therapeutic PMBC"*, and may be used in targeting an active drug or a pro-drug to PNOM- cells, and can therefore be useful to achieve targeting of treatment to foci of disease in medical disorders characterized by the presence of PNOM-cells as defined above.

The association between the medicinally-useful drug and the PMBC wherein it is comprised may be by covalent binding, by non-covalent binding (e.g., electrostatic forces) or by formation of carrier particles (such as liposomes) comprising the drug and having on their surface a PMBC which targets the complex to the PNOM-cells.

The purpose of a therapeutic PMBC is to direct the drug comprised within selectively to PNOM-cells and to tissues and organs being foci of disease, thereby comprising said cells. Once the drug reaches the target it should be able to exert its physiological activity, either when still being part of the PMBC-conjugate, after disconnecting from the PMBC unit (for example by cleavage, destruction, etc., activity of natural enzymes), by phagocytosis of drug-containing liposomes having PMBC on their membrane, or by any other known mechanism.

The drug should be chosen in accordance with the specific disease for which the composition is intended.

For treatment or prevention of diseases which are manifested by initiation or propagation of *abnormal and excessive blood clotting* [e.g., arterial or venous thrombosis, thrombo-embolism, sickle cell diseases, beta-thalassemia, antiphospholipid antibody syndrome, disseminated intravascular coagulation (DIC),

thrombotic thrombocytopenic purpura (TTP), systemic lupus erythematosus], the drug should be a compound which is known to inhibit formation of blood clots, or to dissolve blood clots after they have been formed, such as an antiplatelet agent, heparin, low molecular weight heparin, antagonists of glycoprotein IIB/IIIA, tissue plasminogen activator (tPA), or an inhibitor of a clotting factor, such as an inhibitor of thrombin, or an inhibitor of factor Xa.

Where the disease is manifested by *inappropriate and excessive apoptosis* such as degenerative disorders, neuodegenerative disorders (e.g., Parkinson's disease, Alzheimer's disease, prion disease, ALS, Huntington chorea), AIDS, myelodysplastic syndromes, ischemic or toxic insults, graft cell loss during transplant rejection, the drug should be capable of inhibiting apoptosis. Such drug may be, among others, a caspase inhibitor, a modulator of the Bcl-2 system or an anti-oxidant.

Where disease is an *inflammatory disorder, and / or a disease associated with immune–mediated etiology or pathogenesis*, such as auto-immune disorders for example antiphospholipid antibody syndrome, systemic lupus erythematosus, connective tissue disorders such as rheumatoid arthritis, scleroderma; thyroiditis; dermatological disorders such as pemphigus or erythema nodosum; autoimmune hematological disorders; autoimmune neurological disorders such as myasthenia gravis; multiple sclerosis; inflammatory bowel disorders such as ulcerative colitis; vasculitis, the drug should be an anti-inflammatory drug, or an immuno-modulator drug.

For the treatment or prevention of an unstable atherosclerotic plaque, characterized by thrombosis, inflammation or apoptosis, the drug can be chosen from the above groups of drugs.

The PMBC of the invention may comprise an anticancer drug in order to enhance the efficacy of anticancer protocols. Said enhancement of an anti-cancer protocol is achieved by either:

(1) Targeting of said conjugate to a tumor tissue; such tissue is often characterized by an abnormally excessive apoptotic load (the latter being often correlated to the level of tumor aggressiveness);

(2) Use of two waves of apoptosis: the first wave being achieved by using a chemotherapeutic or radio-therapeutic agent, aimed at initiating an apoptotic process within the tumor; followed by a second wave of apoptosis, in which the anticancer drug is administered as part of a therapeutic PMBC as defined above, capable of targeting the apoptotic cells produced by the first wave. Thus, augmentation of the local concentrations of the anticancer drug within the tumor mass is achieved, with consequent enhancement of the local tumor-killing process, while maintaining relatively lower levels of the drug in non-tumor tissues.

The pharmaceutical composition as well as the diagnostic composition of the invention may be administered by any of the known routes, *inter alia*, oral, intravenous, intraperitoneal, intramuscular, subcutaneous, sublingual, intraocular, intranasal or topical administration, or intracerebral administartion. The carrier should be selected in accordance with the desired mode of administration, and include any known components, e.g. solvents; emulgators, excipients, talc; flavors; colors, etc. The pharmaceutical composition may comprise, if desired, also other pharmaceutically-active compounds which are used to treat the disease, eliminate side effects or augment the activity of the active component.

The present invention also concerns use of a PMBC-conjugate comprising a drug, i.e., a therapeutic PMBC for the preparation of a medicament.

The use according to the present invention is preferably for the preparation of a medicament for the treatment and detection of diseases characterized by presence of PNOM-cells, and / or in diseases in which PNOM-cells have an etiological or a pathogenetic role. Examples of such diseases are given above.

The present invention in accordance with this aspect, still further concerns a method for improvement of treatment of a disease manifested by PNOM-cells, comprising administering to an individual in need of such treatment an effective

amount of a therapeutic PMBC, said therapeutic PMBC comprising a drug being active as a treatment for said disease or a pro-drug which is converted to an active drug in the targeted area. The therapeutic PMBC allows for selective targeting of the drug or the drug to the tissues comprising PNOM-cells, thus augmenting its local concentration, and potentially enhancing its therapeutic effect at the target site. Such medical disorders are those defined above.

The term *"effective amount"* refers to an amount capable of decreasing, to a measurable effect, at least one adverse manifestation of the disease and should be chosen in accordance with the drug used, the mode of administration, the age and weight of the patient, the severity of the disease, etc.

By a third approach of the invention, termed the *"clearance approach"*, the properties of the PMBCs of the invention to bind specifically to PNOM-cells are utilized to clear body fluid of said cells. Preferably, the body fluid is blood or a blood product.

Many surgical or medical interventions requiring extracorporeal circulation are associated with exposure of blood elements to exogenous artificial environment. This often leads to activation of and damage to blood cells, systemic inflammation, and thromboembolic phenomena, potentially having serious clinical consequences, such as neurological dysfunction upon lodging of microemboli in the cerebral blood vessels. It is therefore desirable to detect and remove said damaged, activated or apoptotic cells from blood.

Thus, according to one of its aspects, the present invention concerns a PMBC immobilized on a solid support. Said immobilization may be by direct attachment, either by covalent or non-covalent binding, or by attachment through a spacer. The immobilized PMBC is intended to clear a body fluid from PNOM-cells.

According to another embodiment of the present invention, the solid support features a plurality of beads to which the PMBC are bound. Preferably, the beads are resin-coated beads. Alternatively, the beads may be magnetic beads.

Where the solid support includes a plurality of fibers or micro-capillara, among and/or through which the body fluid flows, the inner and/or outer faces thereof are covered with the PMBC.

The compounds immobilized on a solid support form part of a filter device. Thus in accordance with the clearance approach, the present invention further concerns a filter device comprising a housing containing the PMBC immobilized on said solid support, and a fluid inlet and fluid outlet. Body fluids such as blood or blood products enter the housing through said inlet, come into contact and adhere to the immobilized PMBC contained in the housing. Thus, the body fluid is cleared of circulating cells having perturbed membranes, such as damaged or dying cells, or cleared of larger structures such as emboli having PNOM membranes. Consequently, fluid exiting from said outlet has a reduced content of said PNOM-cells or is essentially devoid of same.

The filter device may form a replaceable, a permanent, or an add-on portion of an extracorporeal circulation apparatus. Thus the present invention also concerns an extracorporeal circulation apparatus comprising said filter device, wherein blood circulating through the apparatus also passes through the device.

Examples of such apparatuses are a cardiopulmonary bypass apparatus; a hemodialysis apparatus; a plasmapheresis apparatus and a blood transfusion apparatus, such as state of the art blood transfusion bags.

The clearance aspect also provides a method for removal of PNOM-cells from a biological fluid, preferably a body fluid such as blood. The method comprising: contacting the body fluid with a PMBC immobilized on a solid support, under conditions and for a time period sufficient for binding of the PNOM-cells to the PMBC, thereby removing at least a portion of said PNOM-cells from the body fluid.

The contact of the body fluid (such as blood or blood-derived product) with the immobilized PMBC may be carried-out by any method known in the art to remove particles from fluid, and in particular by flowing the body fluid through the

filter device of the invention, thereby clearing the blood from at least a portion of the microemboli present therein.

The method of the invention may be used to improve the clinical outcome of procedures involving extracorporeal circulation, such as surgery requiring cardiopulmonary bypass, thereby reducing the risk for thromboembolic events during the procedure, by removal of the potentially harmful PNOM-cells or the structures containing PNOM membranes (notably microemboli) from blood. In practice, the filter device may be placed on the line leading from the extracorporeal circulation machine to the patient, thus performing filtration of PNOM-cells or aggregates out of the blood. Alternatively, blood can be temporarily diverted to a system comprising the filter device for performance of the filtration of the elements containing PNOM membranes, with return of the purified blood to the systemic circulation thereafter. The method may also be used to clear PNOM-cells from the circulation of a patient undergoing hemodialysis, or a patient undergoing plasmapheresis.

The method may also be used to treat stored blood prior to transfusion, so as to minimize the amount of PNOM-cells present therein and thus minimize various complications associated with transfusion of blood. For the treatment of stored blood, said filtration method may be used as part of the processing of blood in the blood bank, or during transfusion to the patient, in which case said filter may be placed on the line leading from the transfusion bag to the patient.

EXAMPLES

Example I:

A method for synthesis of NST-ML-F

a. Preparation of diethyl –2-butyl-2-hydroxymethyl-malonate:

A solution of diethyl butylmalonate in dimethyl formamide (DMF) was added to NaH (1.2 equivalents), the slurry stirred for 2 hours, then cooled and benzyloxymethyl chloride (BOM-Cl) in DMF was added. After stirring for 2 hours

at room temperature the mixture was heated overnight at 50°C. Solvent was removed under reduced pressure. Residue was dissolved in ether and water was added to dissolve the precipitate. Ether layer was washed twice with water and once with brine, dried over $MgSO_4$ and solvent removed *in vacuu*. The crude diester was isolated after flash chromatography.

b. Preparation of diethyl 2-butyl-2-fluoromethyl-malonate:

This was performed according to the procedure of JoAnne Stubbe, Susan Fish and Robert H. Abeles, J. Biol. Chem. 255(1) 239-242, (1980), except that Kryptofix 222 fluoride was used as fluorinating agent.

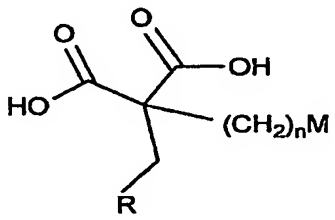
c. Hydrolysis to 2-butyl-2-fluoromethyl-malonic acid:

This was performed according to the above-cited reference.

Example II

Mechanism of action of the compounds of the invention

The active module in the compounds of the invention is the following motif of Formula I (NST-ML-Action Motif):



Wherein R and n are as defined above and M is F, OH, SH or amino.

The NST-ML-Action Motif is designed to correspond to the structural alterations encountered in the plasma membranes of apoptotic cells, which distinguish said membranes from membranes of healthy cells. This complex of membrane alterations comprises:

1. Scrambling of membrane phospholipids, with exposure on the cell surface of phosphatidylethanolamine (PE) and the negatively-charged phosphatidylserine (PS).
2. Exposure of PS on the cell surface leads to a *negative surface electric potential*, and attraction of protons form the bulk to the membrane interface.
3. The increase in the fraction of aminophospholipids (PE and PS) within the the outer leaflet of the membrane results in an *enhancement of the proton currents in the interface of the outer leaflet of the membrane (interfacial proton currents, IPC)*. This enhancement is due to the substantial increase in the number of functionalities amenable to participation in proton transfer reactions, as PE and PS replace phosphatidylcholine (PC) and sphingomeylin (SM) in the outer membrane leaflet. PE comprises a primary amine, while PS comprises a primary amine and a carboxyl group. By contrast, PC and SM comprise each a quaternary ammonium, that bears a permanent positive charge, and thus cannot participate in proton transfer reactions.
4. Apoptotic membranes are characterized by a reduced level of packing of the membrane constituents.

The NST-ML-Action Motif is a *switch moeity*, activated selectively upon its approaching a membrane which features the above characteristics, i.e., the plasma membrane of an apoptotic cell (Figure 1). The Action Motif is highly soluble in acquous solution, due to its having two negatively-charged carboxyl groups (pKa of alkylmalonate is about 5.6 and 2.8), thus having a formal charge of –2 in physiological conditions. However, upon approaching the apoptotic membrane, due to the more acidic surface, and due to the reduction in the dielectric constant of the interfacial environment, which acts to elevate pKa values of the carboxyl groups, *a proton is being caputed by the malonate moeity.*

The capture of the proton by the malonate group neutralizes one of the negative charges, and renders the overall charge of the molecule to –1. Moreover, the capture of the proton further leads to a very unique situation, which includes the following:

1. An acid-anion pair is formed, wherein an exceptionally strong hydrogen bond is formed between the protonated and unprtonated carxoyl groups. This hydrogen bond is strong, symmetrical and stabilized by resonance and tautamerization.
2. This leads to distribution of the negative charge over the four carboxyl atoms, i.e., its being partially delocalized.
3. The strong acid-anion hydrogen bond rigidifies the molecule, creating a bulky, rigid, flat, six-memebered ring, bearing a partially-delocalized negative charge, and comprising pi-electron clouds over the carboxyl bouble bonds.
4. Such an "aromatic" element manifests relatively favorable penetration into the membrane interface. However, its bulky, rigid structure directs its binding selectively to loosely packed emebranes, i.e., apoptotic memebranes, rather than binding to highly-packed membranes such as the plasma membranes of healthy cells. These steric features therefore promote selectivity in binding to the apoptotic membranes.
5. Upon the selective penetration of the single-protonated malonate into the membrane interface, it becomes subjected to the enhanced interfacial proton currents, and to extensive interfacial network of hydrogen bonds. The probability of its acquiring a second proton, neurtralization of charge and formation of further acid-anion pairs with adjacent phospholipid molecules is therefore markedly enhanced. These events further act to stabilize the binding of the molecule to the interface.

6. The penetration of the protonated malonate moiety into the interface and the stabilization of its binding there allow the alkyl chain R to traverse the membrane interface, reach its optimal binding environment, i.e., the membrane hydrocarbon core, and contributes to the binding energy of the compound through hydorphobic interactions.
7. Moiety $(CH_2)_nM$ acts to tune the pKa of the malonate moiety to the optimal range, by a potential electron withdrawing effect of M (e.g., in the case that M is F or O), exerted on the malonate carboxyl groups in dependence on the length of the alkylene linker $[(CH_2)_n]$.

The NST-ML-Action Motif is being utilized for useful diagnostic or therapeutic purposes, through its binding to a marker for imaging or a therapeutic drug (moiety D in Formula I) through a hydrocarbon linker $[(CH_2)_m$ of Formula I]. *The NST-ML-Action Motif thus acts as a targeting moeity*, allowing selective targeting of the marker for imaging or drug attached to it to cells and tissues inflicted by cell death, paricularly apoptosis, or platelet activation and thrombosis.

Figure 1 demonstrates NST-ML-F (formula II), and describes the three stages in the approach of the compound and its binding to the PNOM membrane:

A: The compound is in solution, both carboxyl groups are deprotonated, i.e., negatively-charged, and the compound is highly soluble.

B: Upon approaching the negatively-charged apoptotic membrane, the compound acquires a proton. An anion-acid dimer is formed, thus creating a stable six-membered, resonance-stabilized ring, which penetrates the membrane interface region. The bulky, rigid ring structure assists in selectivity, since its steric features favor binding to the more loosely-packed plasma membrane of the apoptotic cell.

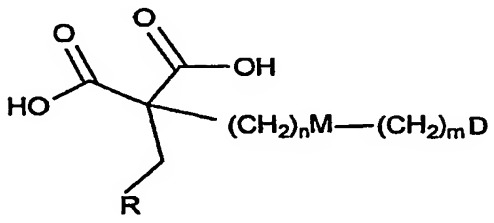
C: Upon compound penetration into the membrane interface, it is subjected to the interfacial network of hydrogen bonds, and to the augmented interfacial

proton currents. The resultant protonation and hydrogen bonding further acts to stabilize the binding of the compound to the interface (arrows). The alkyl chain further contributes to the binding energy by creating hydrophobic interactions with the membrane hydrocarbon core.

CLAIMS:

1. A method for selective targeting of a compound to a cell undergoing *p*erturbation of the *n*ormal *o*rganization of its plasma *m*embrane (PNOM-cell), comprising the steps of:

contacting a cell population comprising said PNOM-cell with a compound or a conjugate comprising said compound wherein said compound is represented by the structure set forth in formula (I):



(I)

or pharmaceutically acceptable salts, metal chelates, solvates and hydrates of the compound represented by the structure as set forth in formula (I), and solvates and hydrates of said salts; wherein,

R represents hydrogen, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10} linear or branched alkyl, linear or branched hydroxy-alkyl; or linear or branched fluoro-alkyl;

n and m each stands for an integer of 0, 1, 2, 3 or 4; n and m can be the same or different;

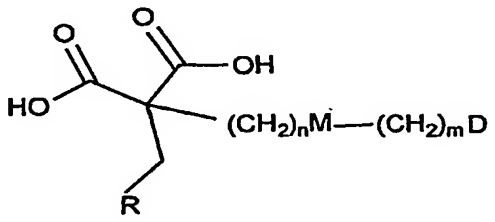
M is selected from null, -O-, -S-, -C(O)NH-, and -NU, wherein U stands for a null, hydrogen, C_1, C_2, C_3, or C_4 alkyl;

D is selected from hydrogen; a drug to be targeted to a cell undergoing *p*erturbation of the *n*ormal *o*rganization of its plasma *m*embrane (PNOM-cell); and a marker for diagnostics selected from a marker for imaging and a metal chelate; said marker for imaging being selected from the group comprising a fluorescent label, a radio-label, a marker for X-ray, a marker for MRI, a marker for PET scan and a label capable of undergoing an enzymatic reaction that produces a detectable color; and

where the above alkylene groups in formula (I) bound to M or D may be each substituted at each occurrence by a group selected from amino, F, OH and SH, thereby selectively targeting said compound to said PNOM-cell within said cell population.

2. A method of detecting a PNOM-cell within a cell population, said method comprising:

(i) contacting the cell population with a compound or a conjugate comprising said compound wherein said compound is represented by the structure set forth in formula (I):



(I)

or pharmaceutically acceptable salts, metal chelates, solvates and hydrates of the compound represented by the structure as set forth in formula (I), and solvates and hydrates of said salts; wherein,

R represents hydrogen, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10} linear or branched alkyl, linear or branched hydroxy-alkyl; or linear or branched fluoro-alkyl.

n and m each stands for an integer of 0, 1, 2, 3 or 4; n and m can be the same or different ;

M is selected from null, -O-, -S-, -C(O)NH-, and -NU, wherein U stands for a null, hydrogen, C_1, C_2, C_3, or C_4 alkyl, ;

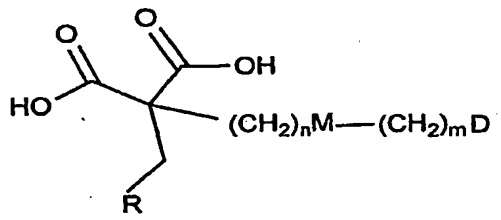
D is selected from hydrogen; a drug to be targeted to a cell undergoing *p*erturbation of the *n*ormal *o*rganization of its plasma *m*embrane (PNOM-cell); and a

marker for diagnostics selected from a marker for imaging and a metal chelate; said marker for imaging being selected from the group comprising a fluorescent label, a radio-label, a marker for X-ray, a marker for MRI, a marker for PET scan and a label capable of undergoing an enzymatic reaction that produces a detectable color; and where the above alkylene groups in formula (I) bound to M or D may be each substituted at each occurrence by a group selected from amino, F, OH and SH; and

(ii) determining the amount of said compound bound to said cells, wherein a significant amount of said compound bound to a cell indicates its being a PNOM-cell.

3. A method for detecting of PNOM-cells in a patient or an animal, comprising:

(i). administering a compound or a conjugate comprising said compound wherein said compound is represented by the structure set forth in formula (I):



(I)

or pharmaceutically acceptable salts, metal chelates, solvates and hydrates of the compound represented by the structure as set forth in formula (I), and solvates and hydrates of said salts; wherein,

R represents hydrogen, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10} linear or branched alkyl, linear or branched hydroxy-alkyl; or linear or branched fluoro-alkyl.

n and m each stands for an integer of 0, 1, 2, 3 or 4; n and m can be the same or different ;

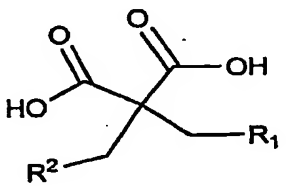
M is selected from null, -O-, -S-, -C(O)NH-, and -NU, wherein U stands for a null, hydrogen, C_1, C_2, C_3, or C_4 alkyl,;

D is selected from hydrogen; a drug to be targeted to a cell undergoing *p*erturbation of the *n*ormal *o*rganization of its plasma *m*embrane (PNOM-cell); and a marker for diagnostics selected from a marker for imaging and a metal chelate; said marker for imaging being selected from the group comprising a fluorescent label, a radio-label, a marker for X-ray, a marker for MRI, a marker for PET scan and a label capable of undergoing an enzymatic reaction that produces a detectable color; where the above alkylene groups in formula (I) bound to M or D may be each substituted at each occurrence by a group selected from amino, F, OH and SH; and

(ii) imaging the human or animal, so as determine the amount of said compound bound to cells, wherein a significant amount of said compound bound a cell indicates its being a PNOM-cell.

4. A method for selective targeting of a compound to a cell undergoing *p*erturbation of the *n*ormal *o*rganization of its plasma *m*embrane (PNOM-cell), comprising the steps of:

(i). contacting a cell population comprising said PNOM-cell with a compound or a conjugate comprising said compound wherein said compound is represented by the structure as set forth in formula (II):



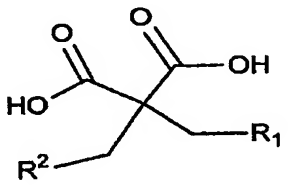
II

including pharmaceutically acceptable salts hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (II) and solvates and hydrates of said salts; wherein R^1 is hydrogen or C_1, C_2, C_3, C_4, or C_5 linear or branched alkyl, and R^2 is hydrogen or C_1, C_2, C_3, C_4, or C_5 linear or branched alkyl, hydroxy-alkyl or fluoro-alkyl;

(ii). thereby selectively targeting said compound to said PNOM-cell within said cell population.

5. A method of detecting a PNOM-cell within a cell population, said method comprising:

(i) contacting the cell population with a compound or a conjugate comprising said compound wherein said compound is represented by the structure as set forth in formula (II):



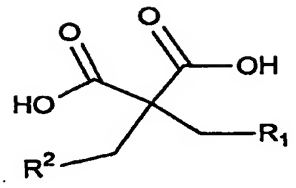
II

including pharmaceutically acceptable salts hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (II) and solvates and hydrates of said salts; wherein R^1 is hydrogen or C_1, C_2, C_3, C_4, or C_5 linear or branched alkyl, and R^2 is hydrogen or C_1, C_2, C_3, C_4, or C_5 linear or branched alkyl, hydroxy-alkyl or fluoro-alkyl; and

(ii) determining the amount of said compound bound to said cells, wherein a significant amount of said compound bound to a cell indicates its being a PNOM-cell.

6. A method for detecting of PNOM-cells in a patient or an animal, comprising:

(i). administering a compound or a conjugate comprising said compound wherein said compound is represented by the structure as set forth in formula (II):

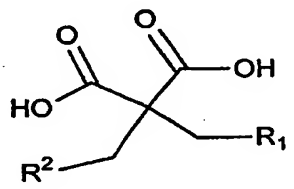


II

including pharmaceutically acceptable salts hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (II) and solvates and hydrates of said salts; wherein R^1 is hydrogen or C_1, C_2, C_3, C_4, or C_5 linear or branched alkyl, and R^2 is hydrogen or C_1, C_2, C_3, C_4, or C_5 linear or branched alkyl, hydroxy-alkyl or fluoro-alkyl; and

(ii) imaging the human or animal, so as determine the amount of said compound bound to cells, wherein a significant amount of said compound bound a cell indicates its being a PNOM-cell.

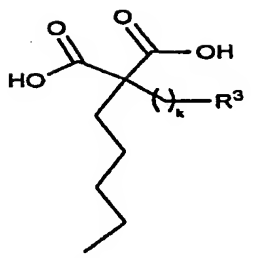
7. A compound represented by the structure as set forth in formula (II):



II

including pharmaceutically acceptable salts hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (II) and solvates and hydrates of said salts; wherein R^1 is hydrogen or C_1, C_2, C_3, C_4, or C_5 linear or branched alkyl, and R^2 is a C_1, C_2, C_3, C_4, or C_5 fluoro-alkyl.

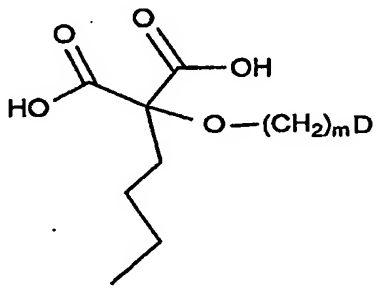
8. A compound represented by the structure as set forth in formula (III):



III

including pharmaceutically acceptable salts hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (II) and solvates and hydrates of said salts; wherein R^3 is hydroxyl or F and k is an integer selected from 1, 2, 3, 4 and 5.

9. A compound represented by the structure as set forth in formula (IV):

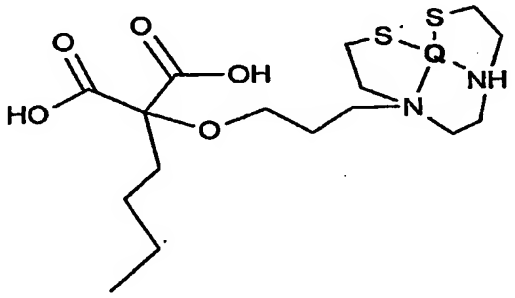


IV

including pharmaceutically acceptable salts hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (IV) and solvates and hydrates of said salts; wherein m stands for an integer of 0, 1, 2, 3 or 4; and D is selected from hydrogen; a drug to be targeted to a cell undergoing *p*erturbation of the *n*ormal *o*rganization of its plasma *m*embrane (PNOM-cell); and a marker for diagnostics selected from a marker for imaging and a metal chelate; said marker for imaging being selected from the group comprising a fluorescent label, a radio-label,

a marker for X-ray, a marker for MRI, a marker for PET scan and a label capable of undergoing an enzymatic reaction that produces a detectable color.

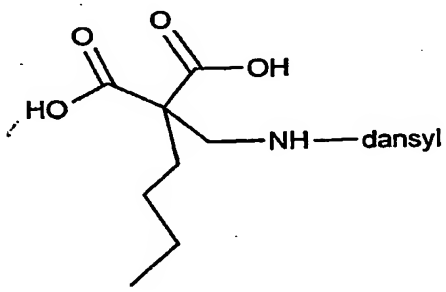
10. A compound represented by the structure as set forth in formula (V):



(IV)

including pharmaceutically acceptable salts hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (V) and solvates and hydrates of said salts, wherein m and Q is a metal atom selected from Technetium, oxo-Technetium, Rhenium and oxo-Rhenium.

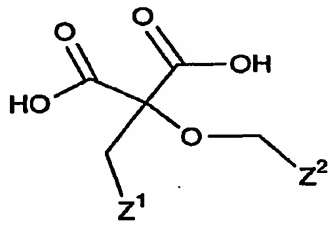
11. A compound represented by the structure as set forth in formula (VI),



V

including pharmaceutically acceptable salts hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (V) and solvates and hydrates of said salts.

12. A compound represented by the structure as set forth in formula (VII):



VII

including pharmaceutically acceptable salts hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (VII) and solvates and hydrates of said salts; wherein Z^1 and Z^2 are each selected from hydrogen and C_1, C_2, C_3, C_4, C_5 alkyl, hydroxy-alkyl or fluoro-alkyl; Z groups may be the same or different.

13. A compound according to claims 1-6 and 7-12, comprising or linked to a marker for imaging, selected from the radioisotopes of the metal ions Tc, Tc=O, In, Cu, Ga, Xe, Tl, Re and Re=O for radio-isotope scan.

14. A compound according to claims 7-12, comprising or linked to a marker for imaging selected from the radioisotopes ^{123}I and ^{131}I for radio-isotope scan.

15. A compound according to claims 7-12, comprising or linked to a marker for imaging selected from Gd(III), Fe(III), Fe_2O_3, Fe_3O_4 and Mn(II) for MRI scan.

16. A compound according to claims 7-12, comprising or linked to a marker for imaging selected from ^{18}F, ^{15}O, ^{18}O, ^{11}C, ^{13}C, ^{124}I, ^{13}N, ^{75}Br and ^{64}Cu for positron emission tomography (PET) scan.

17. A compound according to claims 7-12, comprising or linked to a marker for imaging selected from Tc-99m and In-111 for SPECT, ^{18}F for PET and Gd for MRI.

18. A method for selective targeting of a compound according to claims 7-16 to a cell undergoing *p*erturbation of the *n*ormal *o*rganization of its plasma *m*embrane (PNOM-cell), comprising the steps of:

(i). contacting a cell population comprising said PNOM-cell with a compound according to claims 7-16 or a conjugate thereof, or pharmaceutically acceptable salts, metal chelates, solvates thereof;

(ii). thereby selectively targeting said compound to said PNOM-cell within said cell population.

19. A method of detecting a PNOM-cell within a cell population, said method comprising:

(i) contacting the cell population with a compound according to claims 7-16 or a conjugate thereof, or pharmaceutically acceptable salts, metal chelates, solvates thereof; and

(ii) determining the amount of said compound bound to said cells, wherein a significant amount of said compound bound to a cell indicates its being a PNOM-cell.

20. A method for detecting of PNOM-cells in a patient or an animal, comprising:

(i) administering a compound according to claims 7-16 or a conjugate thereof, or pharmaceutically acceptable salts, metal chelates, solvates thereof; and

(ii) imaging the human or animal, so as determine the amount of said compound bound to cells, wherein a significant amount of said compound bound a cell indicates its being a PNOM-cell.

21. The method according to Claims 1-6 and 18-20, wherein the PNOM cell is a cell undergoing a death process, an apoptotic cell, or an activated platelet.

22. The method according to Claims 1-6 and 18-20, wherein the PNOM cell is within whole body, organ, tissue, tissue culture, body fluid or cell culture.

23. A pharmaceutical composition for targeting of a drug to tissues inflicted by apoptosis or blood clotting in a patient, comprising a compound according to any of the structures as set forth in formulae I, II, III, IV, V, VI, VII wherein said compound comprises or being linked to said drug.

24. A pharmaceutical composition according to Claim 23, wherein the drug is selected from: an inhibitor of apoptosis, a cytotoxic agent, an anti-platelet

agent, an anticoagulant, a fibrinolytic agent, an anti-inflammatory drug and an immuno-modulator drug.

25. A method according to Claims 2, 3, 6, 7, 19 and 20 for the detection of cell death in a disease characterized by occurrence of apoptosis.

26. A method according to Claims 2, 3, 6, 7, 19 and 20 for the detection of activated platelets is a disease characterized by blood clotting.

27. A method according to Claims 2, 3, 6, 7, 19 and 20 for detection of apoptosis within a tumor, for monitoring of aggressiveness of a tumor or for detection of metastases of a tumor.

28. A method according to Claims 2, 3, 6, 7, 19 and 20 for monitoring the response of a tumor to anti-cancer treatment.

29. A method according to Claims 2, 3, 6, 7, 19 and 20 for monitoring of apoptosis in normal tissues during anticancer treatment, being and adverse effect of said treatment.

30. A method according to Claims 2, 3, 6, 7, 19 and 20 for monitoring of survival of a grafted organ after transplantation.

31. A method according to Claims 2, 3, 6, 7, 19 and 20 for monitoring of response to cytoprotective therapy in a disease characterized by excessive apoptosis.

32. A method according to claims 1-6, wherein the marker for imaging is selected from the radioisotopes of the metal ions Tc, Tc=O, In, Cu, Ga, Xe, Tl, Re and Re=O for radio-isotope scan.

33. A method according to claims 1-6, wherein the marker for imaging is selected from the radioisotopes ^{123}I and ^{131}I for radio-isotope scan.

34. A method according to claims 1-6, wherein the marker for imaging is selected rom Gd(III), Fe(III), Fe_2O_3, Fe_3O_4 and Mn(II) for MRI scan.

35. A method according to claims 1-6, wherein the marker for imaging is selected from ^{18}F, ^{15}O, ^{18}O, ^{11}C, ^{13}C, ^{124}I, ^{13}N, ^{75}Br and ^{64}Cu for positron emission tomography (PET) scan.

36. A method according to claims 1-6, wherein the marker for imaging is selected from Tc-99m and In-111 for SPECT, ^{18}F for PET and Gd for MRI.

Abstract

The present invention relates to compounds that selectively bind to cells undergoing perturbations and alterations of their normal plasma membrane organization, such as cells undergoing apoptosis and activated platelets. The invention further provides methods for utilizing said compounds in medical practice, for diagnostic and therapeutic purposes.

Figure I